DATA EVALUATION RECORD
WHOLE SEDIMENT ACUTE TOXICITY INVERTEBRATES, FRESHWATER
OPPTS Guideline 850.1735

1. **CHEMICAL:** Bifenthrin PC Code: 128825

2. **TEST MATERIAL:** VCP-03 Purity: 19.61% ai

3. **CITATION:**

Authors:	Thomas, S.T., *et al.*
Title:	Bifentrhin: A 10-Day Acute Toxicity Test with the Freshwater Amphipod (*Hyalella azteca*) Using Spiked Sediment.
Study Completion Date:	August 14, 2014
Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, MD 21601
Sponsor:	Vive Crop Protection, Inc. 700 Bay Street Suite 1100 Toronto, Ontario Canada M5G 1Z6
Laboratory Report ID:	754A-101
MRID No.:	49462202
DP Barcode:	423368

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, CSS-Dynamac
Signature: Christie E. Padova **Date:** 01/22/15

APPROVED BY: Teri S. Myers, Senior Scientist, CDM Smith
Signature: Teri S Myers **Date:** 01/27/15

5. **APPROVED BY:**
Signature: **Date:** 03/27/15

6. **STUDY PARAMETERS:**

Age of Test Organism:	*ca.* 9 days old
Definitive Test Duration:	10 days
Study Method:	Flow-through
Type of Concentrations:	Mean-measured sediment, bulk and OC-normalized

7. CONCLUSIONS:

Results Synopsis:

In terms of mean-measured sediment concentrations:

Survival:
LC_{50}: 276 ng ai/kg 95% C.I.: 238 to 312 ng ai/kg
Probit slope: 4.64 95% C.I.: 3.6 to 5.69
NOAEC: 126 ng ai/kg
LOAEC: 227 ng ai/kg

Growth:
NOAEC: 126 ng ai/kg
LOAEC: 227 ng ai/kg

In terms of OC-normalized mean-measured sediment concentrations:

Survival:
LC_{50}: 9857 ng ai/kg TOC 95% C.I.: 8500 to 11,143 ng ai/kg TOC
Probit slope: 4.64 95% C.I.: 3.6 to 5.69
NOAEC: 4500 ng ai/kg TOC
LOAEC: 8100 ng ai/kg TOC

Growth:
NOAEC: 4500 ng ai/kg TOC
LOAEC: 8100 ng ai/kg TOC

8. ADEQUACY OF THE STUDY:

A. Classification: This study is classified as SUPPLEMENTAL. The survival endpoints are considered scientifically sound and can be used quantitatively in risk assessment. The growth endpoints are considered supplemental and can be used qualitatively for risk characterization only.

B. Rationale: Analysis of two matrix blank samples (Days 0 and 10) revealed a peak eluting with approximately the same retention time as bifenthrin in the sediment, at concentrations of 43.8 and 35.0 ng ai/kg. Measured concentrations of bifenthrin (36.8 to 68.2 ng ai/kg) were also detected in the negative and solvent control samples at days 0 and 10. The maximum value of these residues corresponds to approximately 50% of the reported NOAEC, 30% of the reported LOAEC, and 24% of the LC_{50} from this study. It is not clear if this represented contamination of samples or controls (or some combination of

both). However, the fact that it occurred on multiple occasions (6 samples total) suggests that this contamination was a systematic problem and not a spurious result. Survival in the two controls did not appear to be negatively impacted (91-93%). However, growth in controls (0.085 – 0.11 mg-d.w./amphipod) at 10 days was near (or slightly below) the lower bounds of growth reported from historical controls at this lab (0.098-0.22 mg-d.w./amphipod). Therefore, this reviewer cannot conclusively discount the potential impact of this contamination on growth of control organisms. If contamination was negatively impacting growth of control amphipods even by a relatively small margin, then a lower NOAEC could have been observed in this study. Therefore, this study is considered supplemental because of uncertainty associated with the growth NOAEC and LOAEC endpoints determined from this study. The survival results can be used quantitatively in risk assessment.

C. Repairability: Evidence would be needed to conclusively determine that contamination was limited to sampling/analytical procedures and not control contamination in order to upgrade this study.

9. MAJOR GUIDELINE DEVIATIONS:

- Bifenthrin was detected in matrix blank and control (negative and solvent) samples on Days 0 and 10.

10. SUBMISSION PURPOSE: New Use

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *H. azteca* or *Chironomus tentans*	*Hyalella azteca*

Guideline Criteria	Reported Information
Life Stage: For *C. tentans*: third instar (9-11 days old). The instar stage of midges must be confirmed by head capsule width (approx. 0.38 mm). For *H. azteca*: 7- to 14-day old amphipods must be produced. If growth is also an endpoint, a narrower range, such as 1- to 2-day old amphipods should be used.	 *H. azteca*: *ca.* 9 days old At study initiation, the dry weight of a sub-population of 20 amphipods averaged 0.035 mg dw/amphipod.
Supplier Brood stock can be obtained from laboratory, commercial, or government sources (sources obtained from the wild should be avoided unless cultured through several generations in the laboratory).	Aquatic BioSystems, Inc., Fort Collins, CO
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period: The required culture and testing temperature is 23°C. The test organisms should be cultured in the same water to be used for testing.	The organisms were held in water from the same source as the water used during the test. During the 14-day holding period immediately preceding the test, water temperature ranged from 21.8 to 23.6°C, pH ranged from 8.2 to 8.6, and dissolved oxygen ranged from 7.5 to 8.7 mg/L.
Feeding:	TetraMin® flake food
Pretest Mortality: A group of organisms should not be used if they appear unhealthy, discolored (e.g. <20%	Amphipods appeared normal during the holding period.

Guideline Criteria	Reported Information
mortality 48 h before the beginning of a test).	

C. Test System

Guideline Criteria	Reported Information
Source of dilution water (overlying water) and sediment: Soft reconstituted water or water from a natural source. Tap water is acceptable if it is dechlorinated, deionized, and carbon filtered, but its use is not encouraged. Uncontaminated natural sediment is recommended.	Moderately-hard freshwater was obtained from an on-site laboratory well and sand-filtered and aerated. Prior to use, the water was again filtered (0.45 µm). Formulated sediment was prepared similar to that described in OECD Guideline 218 by mixing the following components: *ca.* 0.01% humic acid, 0.99% dolomite, 5% alpha-cellulose, 14% silt and clay (kaolin clay), and 80% industrial quartz sand. The dry constituents were mixed in a PK Twinshell mixer for *ca.* 20 minutes, and were stored at ambient conditions until needed. The results of periodic analyses to measure selected organic and inorganic constituents were provided for well water (sampled 12/19/13) and formulated sediment (sampled 12/16/13).
Does water support test animals without observable signs of stress?	Yes.
Quality Of Water If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 µg/L	There were no apparent problems with water quality. The TOC of the well water was <2 mg/L as C, as measured once during the month preceding the study. Measurements of particulate matter, COD, and residual chlorine were not provided.

Guideline Criteria	Reported Information
Water Temperature 23°C for both species. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1°C and 3°C, respectively.	22.0 to 22.7°C (daily monitoring) 22.87 to 23.31°C (continuous monitoring)
pH Should not vary more than 50%. Survival is best at pH >6.5 for *C. tentans*.	7.5 to 8.3
Dissolved Oxygen Maintained between 40 and 100%.	7.0 to 8.6 mg/L (≥82% saturation)
Total Hardness Should not vary more than 50%. *H. azteca* are sensitive to hardness (e.g., they are not found in waters with calcium at <7 mg/L and DO at <2 mg/L).	124 to 144 mg/L as $CaCO_3$
Conductivity Should not vary more than 50%.	331 to 397 µS/cm
Sediment Characterization All sediment must be characterized for: pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content.	Particle distribution – 81% sand, 8% silt, and 11% clay USDA Textural Class – Loam sand Organic matter content – 4.9% Organic carbon content – 2.8% Moisture at 1/3 Bar – 11.8% pH – 6.7 CEC – 2.6 meq/100 g Bulk density – 1.15 g/cm^3
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	None reported

Guideline Criteria	Reported Information
Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.	Test substance: VCP -03 (Bifenthrin LFF Suspension Concentrate) Common name: bifenthrin IUPAC name: 2-methylbiphenyl-3-ylmethyl (1*RS*,3*RS*)-3-[(*Z*)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropanecarboxylate CAS name: (2-methyl[1,1′-biphenyl]-3-yl)methyl (1*R*,3*R*)-*rel*-3-[(1*Z*)-2-chloro-3,3,3-trifluoro-1-propen-1-yl]-2,2-dimethylcyclopropanecarboxylate CAS No.: 82657-04-3 Description: liquid Lot No.: 36-205-47 Purity: 19.61% ai Storage: ambient temperature
Stock Solutions Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.	A 10 µg ai/mL primary stock solution was prepared in acetone. The primary stock solution was then serially diluted with acetone to prepare the five secondary stocks (63, 125, 250, 500 and 1000 ng ai/mL). Negative and solvent control groups were included in the test.

Guideline Criteria	Reported Information
Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.	Nominal test concentrations were prepared on a dry weight (dw) sediment basis and were corrected for the percent active ingredient. A 1.3-mL volume of the appropriate dosing solution was added to 65 g of clean sand, and hand-mixed with a stir rod. The dosed sand premixes were then placed in a fume hood for *ca.* 1 hour to allow the solvent to evaporate. The premix was then added to 585 g of untreated sediment and mixed on a rotary mixer for 1.8 hours. Finally, 650 g of untreated sediment was added to achieve a final weight of 1300 g, and the batch sediments were mixed on a rotary mixer *ca.* 41.5 hours. The range of concentrations (63 to 1000 mg/kg dw sediment) was selected to establish the LC_{50} and NOAEC/LOAEC.
Test Aquaria 1. Material: Glass or stainless steel or perfluorocarbon plastics. 2. Size: 300 ml high-form lipless beakers containing 100 ml of sediment and 175 ml of overlying water.	1. Glass and stainless steel mesh 2. 300-mL beakers (compartments) with two stainless steel mesh-covered holes on either top side. Test compartments contained approximately 100 mL (depth of *ca.* 3.0 cm) of treated sediment and *ca.* 150 to 175 mL of overlying water (depth of *ca.* 6.1 cm).

Guideline Criteria	Reported Information
Type of Dilution System Daily renewal or a flow-through system may be used.	Flow-through The test compartments were indiscriminately placed into a temperature-control water bath within a diluter tank (with one control or treatment group per tank). The water level in the test compartments (i.e., beakers) was maintained by the water levels in the diluter tanks.
Flow Rate 2 volume changes/day	At least 2 volume additions/day
Aeration Dilution water should be vigorously aerated prior to use so that dissolved oxygen in the overlying water remains above 40% saturation.	None reported
Photoperiod 16 hours light, 8 hours dark at 500 to 1000 lux.	16 hours light/8 hours dark, with 30-minute transition periods. Light intensity at test initiation was 616 lux at the surface of the water over one representative test chamber.
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used,it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	Acetone, 0.001 mL/g sediment

D. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment.	Based upon the results of a pore water equilibration trial (see Reviewer's Comments), the prepared sediment/water systems were allowed to acclimate for 2 days (*ca.* 49 hours) prior to introduction of the test organisms.
Renewal of Overlying Water: Renewal of the overlying water should be conducted on day -1 prior to the addition of organisms or food on day 0. For flow-through systems, the flow rates should not vary by more than 10% between any two chambers at any time. Proper operation should be verified by calibration prior to test initiation.	The dilution water was delivered directly into the test compartments, passively forcing out the water through the holes in the sides of the beakers. The diluter was calibrated prior to test initiation.
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	Amphipods were impartially assigned one and two at a time into transfer chambers until each chamber contained ten organisms. Each group of ten organisms was then impartially assigned and transferred to a test compartment. All transfers were made beneath the water surface using wide-bore pipettes.

Guideline Criteria	Reported Information
Range Finding Test A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment**.**	Preliminary toxicity assessment • Non-GLP, 10-day exposure; same methods as used in definitive study • nominal levels of 0 (negative and solvent controls), 8.1, 27, 90, 300 and 1000 ng ai/kg • 7- to 14-day old amphipods; four replicates per level, each containing 10 organisms • survival averaged 95 (negative control), 98 (solvent control), and 68, 80, 83, 68 and 10%, respectively • dry weight averaged 0.11 (negative control), 0.14 (solvent control), 0.13, 0.14, 0.16 and 0.23 mg per amphipod, respectively • treatment-related effect indicated for survival and growth at the 1000 ng ai/kg level (visual interpretation of the data)
Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.	Test vessels were observed daily for abnormal behavior.
Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.	0 (negative control), 0 (solvent control), 63, 125, 250, 500 and 1000 ng ai/kg dw sediment
Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.	80 amphipods per level, with 10 amphipods per replicate chamber and eight biological replicates per level An additional two replicates per level were maintained for analytical purposes (Days 0 and 10).

Guideline Criteria	Reported Information
Test organisms randomly or impartially assigned to test vessels?	Yes
Feeding *C. tentans* in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin7 suspension daily. *H. azteca* may be fed with a mixture of yeast, Cerophy., and trout chow (YCT) at a rate of 1.5 mL daily per test chamber. A drop in DO levels below 2.5 mg/L may indicate over-feeding and feeding should be suspended in all treatments until DO levels increase.	Amphipods were fed 1 mL of a TetraMin® flake food suspension on Days 0 to 9.
Water Parameter Measurements Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of the test. DO should be measured daily. Temperature should be measured daily in one test chamber from each treatment. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1 and 3°C, respectively.	Hardness, alkalinity, specific conductance and ammonia were measured in composite overlying water samples collected from each control level and the highest treatment level at test initiation and termination. pH was measured in overlying water from one alternating replicate from all levels on Days 0, 3, 7, and 10. DO and temperature were measured in overlying water from one alternating replicate from all levels daily. Temperature was also continuously monitored in the negative control diluter tank.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	Sediment, pore water and overlying water from all levels were analyzed for bifenthrin on Days 0 and 10 (see Reviewer's Comments). Pore water was isolated from sediment using centrifugation at *ca.* 3000 rpm for *ca.* 10 minutes.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was performed in compliance with GLP standards as published by the U.S. EPA (40 CFR Parts 160 and 792), OECD [ENV/MC/CHEM(98)17], and Japan MAFF (11 NohSan, Notification No. 6283, 1999), with the following exceptions: periodic analyses of water and sediment for potential contaminants, and the stability of the test substance under the conditions of storage at the test site.
Control Criteria Was control mortality <20%? Were control *C. tentans* an average size of ≥0.6 g?	 Negative control – 7% Solvent control – 9% N/A
Percent Recovery of Chemical:	Recovery results of quality control (QC) samples analyzed concurrently (Days 0 and 10) with test samples: Sediment: 115 and 88.2% of nominal Pore water: 124 and 114% of nominal Overlying water: 100 and 95.8% of nominal
Data Endpoints - Survival - Dry weight (determined by pooling all living organisms from a replicate and drying at 60 to 90°C to a constant weight) - Body length (amphipod only)	 - Survival - Dry weight
Raw data included?	Yes

Effects Data (Reviewer and Author Reported)

Toxicant Concentration				Mean No. Surviving Amphipods (± SD)	Percent Reduction (%) #	Mean Individual Dry Weights (mg ± SD)	Percent Reduction (%) #
Nominal (ng ai/kg)	Mean-Measured						
	Sediment (ng ai/kg)	Pore Water (ng ai/L)	Overlying Water (ng ai/L)				
Negative Control	<LOQ[a]	<LOQ[a]	<LOQ[a]	9.3 ± 0.89	---	0.085 ± 0.03	---
Solvent Control	<LOQ	<LOQ	<LOQ	9.1 ± 0.99	---	0.11 ± 0.04	---
Pooled Control	---	---	---	9.2 ± 0.91	---	0.099 ± 0.04	---
63	85	<LOQ	<LOQ	8.6 ± 1.19	6.1	0.091 ± 0.04	-7.4
125	126	<LOQ	<LOQ	9.0 ± 1.60	2.0	0.11 ± 0.04	-34
250	227	1.00	<LOQ	5.6 ± 1.92	39*#	0.054 ± 0.02	37*#
500	505	4.02	<LOQ	1.1 ± 0.99	88*#	0.21 ± 0.07	61 [b]
1000	804	16.2	<LOQ	0.13 ± 0.35	99*#	---[b]	---[b]

[a] The LOQ for sediment analysis was 20.0 ng ai/kg; the LOQ for pore water analysis was 10.0 ng ai/L, and the LOQ for overlying water analysis was 5.00 µg ai/L.

[b] Due to the large reduction in survival of this treatment and potential bias on growth of the few surviving organisms, results regarding organism growth in this treatment are not considered appropriate for statistical analysis.

* Author reported statistically-significant difference ($p<0.05$) from the pooled control (Bonferroni t-test).

Reviewer reported statistical significance and % difference from negative control.

Other Significant Results:

Biological: The frequency of sediment avoidance (e.g., organisms leaving the sediment, on the surface of the sediment, climbing the walls of the test compartments, etc.) was increased at the 500 and 1000 ng ai/kg treatment groups and considered to be a treatment-related effect.

Survival after 10 days averaged 9.3 and 9.1 amphipods/replicate for the negative and solvent control levels, respectively, and 8.6, 9.0, 5.6, 1.1 and 0.13 amphipods/replicate for the nominal 63, 125, 250, 500 and 1000 ng ai/kg sediment levels, respectively, corresponding to percent reductions of 6.1, 2.0, 39, 88 and 99% relative to the pooled control (9.2 amphipods/replicate). Differences were statistically-significant compared to the pooled control at the 250, 500 and 1000 ng ai/kg levels ($p<0.05$, Bonferroni t-test). Based on nominal sediment concentrations, the NOAEC for survival was 125 ng ai/kg and the 10-day LC_{50} was 272 ng ai/kg, with 95% confidence intervals of 125 and 500 ng ai/kg.

Mean dry weights were 0.085 and 0.11 mg/amphipod for the negative and solvent control levels, respectively, and 0.091, 0.11, 0.054 and 0.21 mg/amphipod for the nominal 63, 125, 250 and 500 ng ai/kg sediment levels, respectively, corresponding to percent reductions of 10, -13, 47, and -36% relative to the pooled control (0.099 mg/amphipod). Only one organism (out of 80) survived to study termination at the 1000 ng ai/kg level, the dry weight of the organism did not register on the balance, and this level was excluded from statistical analysis. The difference was statistically-significant compared to the pooled control at the 250 ng ai/kg level ($p<0.05$, Bonferroni t-test). The NOAEC for growth was 125 ng ai/kg in terms of nominal sediment concentrations.

Analytical: Results of bifenthrin from dosing stock solutions ranged from 126 to 128% of nominal concentrations. Analysis of two matrix blank samples (Days 0 and 10) revealed a peak eluting with approximately the same retention time as bifenthrin in the sediment, at concentrations of 43.8 and 35.0 ng ai/kg. Measured concentrations of bifenthrin (36.8 to 68.2 ng ai/kg) were also detected in the negative and solvent control samples on days 0 and 10. It was reported that it was not clear if background levels measured in the controls were due to bifenthrin in the control sediment or possible sample contamination, but that the levels were well below the lowest test concentration and the study author indicates that performance of the control organisms did not appear to be impacted (see Reviewer's Comments for further discussion). Sediment sample results were corrected for the background level in the matrix blank samples. In the negative control, solvent control, 63, 125, 250, 500 and 1000 ng ai/kg treatment levels, corrected sediment concentrations were <LOQ (<20.0), 0.714, 59.4, 130, 187, 528 and 844 ng ai/kg, respectively, on Day 0; and 4.67, 22.9, 111, 122, 267, 482 and 763 ng ai/kg, respectively, on Day 10. Mean-measured concentrations were 85, 126, 227, 505 and 804 ng ai/kg, respectively, representing 80 to 135% of nominal levels.

In pore water samples, bifenthrin was below the LOQ (10.0 ng ai/L) at 0 and 10 Days in the negative control, solvent control, and 63 and 125 ng ai/kg treatment levels. At the 250, 500 and 1000 ng ai/kg treatment levels, bifenthrin was 1.16, 4.53 and 12.5 ng ai/L, respectively, on Day 0, and 0.838, 3.51 and 19.9 ng ai/L, respectively, on Day 10. Aside from the Day-10 measurement at the 1000 ng ai/kg level, measured concentrations were extrapolated. Mean-measured pore water concentrations were 1.00, 4.02 and 16.20 ng ai/L at the 250, 500 and 1000 ng ai/kg treatment levels, respectively (reviewer-calculated; see copy of Excel worksheet in Appendix I).

Results from all overlying water samples were below the LOQ (5.00 ng ai/L).

B. Statistical Results

Method: The negative and solvent control survival responses were compared using a Student's t-test. There were no significant differences found between the control groups for survival or growth, and treatment-level data were compared to the performance of the pooled controls.

Survival and growth data were evaluated for normality using the Chi-square test and for homogeneity of variance using Levene's Test (survival) or Bartlett's test (growth). The data were deemed normal with homogeneous variance, and were analyzed using a Bonferroni t-test to identify those treatment levels that were statistically different ($p<0.05$) from the pooled control. The NOAEC and the LOAEC were determined by visual interpretation of the dose-response pattern and statistical analysis of the survival and growth data. The 10-day LC_{50} value and associated 95% C.I. were calculated using binomial probability with nonlinear interpolation.

All statistical analyses were conducted using TOXSTAT Version 3.5. It was reported that since the measured concentrations in the sediment on Days 0 and 10 were slightly elevated - extremely elevated (including measureable amounts of bifenthrin in the control sediments), the results of the test were based on the nominal sediment concentrations.

Survival:
LC_{50}: >272 ng ai/kg 95% C.I.: 125 to 500 ng ai/kg
NOAEC: 125 ng ai/kg
LOAEC: 250 ng ai/kg

Growth:
NOAEC: 125 ng ai/kg
LOAEC: 250 ng ai/kg

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer statistically-verified the results for survival and growth. Negative and solvent control data were compared using a 2-sided, equal variance t-Test; no significant difference was detected. Data were tested for homogeneity of variances using Levene's (dry weight) or Bartlett's test (survival) and for normality using Shapiro-Wilk's test. Data did not satisfy parametric assumptions, so the dry weight NOAEC was determined using the Mann-Whitney U test, while survival data exhibited a decreasing dose-dependent response, so the NOAEC value for this endpoint was determined using the Jonckheere-Terpstra test. Linear regression was used to determine the LC_{50}. These analyses were conducted using CETIS v. 1.8.7.12 with backend settings implemented by EFED on 3/25/14. The reviewer expresses toxicity values using the mean-measured sediment concentrations, along with correction for % TOC.

In terms of mean-measured sediment concentrations:

Survival:
LC_{50}: 276 ng ai/kg 95% C.I.: 238 to 312 ng ai/kg
Probit slope: 4.64 95% C.I.: 3.6 to 5.69
NOAEC: 126 ng ai/kg
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Growth:
NOAEC: 126 ng ai/kg
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In terms of OC-normalized mean-measured sediment concentrations:

Survival:
LC_{50}: 9857 ng ai/kg TOC 95% C.I.: 8500 to 11,143 ng ai/kg TOC
Probit slope: 4.64 95% C.I.: 3.6 to 5.69
NOAEC: 4500 ng ai/kg TOC
LOAEC: 8100 ng ai/kg TOC

Growth:
NOAEC: 4500 ng ai/kg TOC
LOAEC: 8100 ng ai/kg TOC

14. REVIEWER'S COMMENTS:

In terms of statistically-based endpoints, the reviewer's conclusions agreed with the study authors'. However, this reviewer does not agree that the consistent presence of bifenthrin in matrix blanks and control can be conclusively dismissed as not having a detrimental effect on the test organisms (in particular, growth) and the study interpretation.

Analysis of two matrix blank samples (Days 0 and 10) revealed a peak eluting with approximately the same retention time as bifenthrin in the sediment, at concentrations of 43.8 and 35.0 ng ai/kg. Measured concentrations of bifenthrin (36.8 to 68.2 ng ai/kg) were also detected in the negative and solvent control samples at days 0 and 10. The maximum value of these residues corresponds to approximately 50% of the reported NOAEC, 30% of the reported LOAEC, and 24% of the LC_{50} from this study. It is not clear if this represented contamination of samples or controls (or some combination of both). However, the fact that it occurred on multiple occasions (6 samples total) suggests that this contamination was a systematic problem and not a spurious result. Survival in the two controls did not appear to be negatively impacted (91-93%). However, growth in controls (0.085 – 0.11 mg-d.w./amphipod) at 10 days was near (or slightly below) the lower bounds of growth reported from historical controls at this lab (0.098-0.22 mg-d.w./amphipod). Therefore, this reviewer cannot conclusively discount the potential impact of this contamination on growth of control organisms. If contamination was negatively impacting growth of control amphipods even by a relatively small margin, then a lower NOAEC could have been observed in this study. Therefore, this study is considered supplemental because of uncertainty associated with the growth NOAEC and LOAEC endpoints as currently determined. The survival results can be used quantitatively in risk assessment.

This reviewer also notes that the extent of equilibration of spiked sediment was relatively short for this formulation (2 days) compared to the TGAI (usually two weeks). This may reflect the impact of the formulation components on the behavior of the active ingredient, which includes a nano-scale polymer used to encapsulate the active ingredient (bifenthrin). Prior to the definitive study, a non-GLP equilibration test was conducted in order to establish the time required to reach equilibration in the treated sediment (prior to adding the organisms). Six replicate test compartments were prepared at 1000 ng ai/kg bw sediment. The nominal level was selected based on known toxicity data provided by the Sponsor. The samples were held under (proposed definitive) test conditions. One replicate was collected at specified intervals for the analysis of the concentration of bifenthrin in the pore water (e.g. Days 3, 7, 10, 14, 21 and 28). The samples were stored under refrigeration and were analyzed as a batch following collection of the Day 21 sample. Equilibration typically is considered to have occurred when two consecutive samples result in relatively similar measurements. There was no measured bifenthrin in any of the pore water samples. Based on the results of the trial and in consultation with the Sponsor, it was decided that a 2-day

equilibration period was appropriate.

The analytical methods used to analyze the test samples were based upon methods developed by the Wildlife International. Sediment samples (10.0 g) were extracted with acetonitrile amended with anhydrous sodium sulphate; samples were vortexed and centrifuged. Extracts were evaporated to dryness and residues re-dissolved in acetonitrile prior to analysis for bifenthrin using gas chromatography with mass selective detection (GC/MS). Pore and overlying water samples were acidified with phosphoric acid, and then extracted twice with dichloromethane by shaking. The combined organic extracts were evaporated to dryness and residues re-dissolved in acetonitrile prior to analysis using GC/MS. The LOQ was 20.0 ng ai/kg for sediment samples, 10.0 ng ai/L for pore water samples, and 5.00 ng ai/L for overlying water samples.

The experimental phase of the definitive study was conducted from June 16 to 26, 2014.

15. REFERENCES:

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American Society for Testing and Materials. 2005. ASTM Standard E 1706-05. *Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates*.

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APPENDIX I. COPY OF REVIEWER'S MEAN-MEASURED PORE WATER CONCENTRATIONS:

Pore Water

Nominal Conc. ng ai/kg	Day	Measured ng ai/L		Mean-measured ng ai/L
63	0	<LOQ		
	10	<LOQ		#DIV/0!
125	0	<LOQ		
	10	<LOQ		#DIV/0!
250	0	1.16	*	
	10	0.838	*	1.00
500	0	4.53	*	
	10	3.51	*	4.02
1000	0	12.5	*	
	10	19.9		16.20

*Extrapolated value
LOQ = 10.0 ng ai/L

OPPTS 850.1735 Sub-Chronic Sediment (10-d FW) — **Wildlife International**

Analysis ID:	10-7682-0691	**Endpoint:**	Dry Weight	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Jan-15 15:33	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	08-8335-4172	**Test Type:**	Sediment Toxicity 10-d	**Analyst:**	
Start Date:	16 Jun-14	**Protocol:**	OPPTS 850.1735 Sub-chronic Sediment (1	**Diluent:**	Well Water
Ending Date:		**Species:**	Hyalella azteca	**Brine:**	Not Applicable
Duration:	NA	**Source:**	Aquatic Biosystems, CO	**Age:**	9d

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	38.0%	Passes dry weight

Equal Variance t Two-Sample Test

Control	vs	Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		Solvent Blank	-1.57	1.76	0.032	14	0.9306	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.00330625	0.00330625	1	2.46	0.1388	Non-Significant Effect
Error	0.0187875	0.001341964	14			
Total	0.02209375		15			

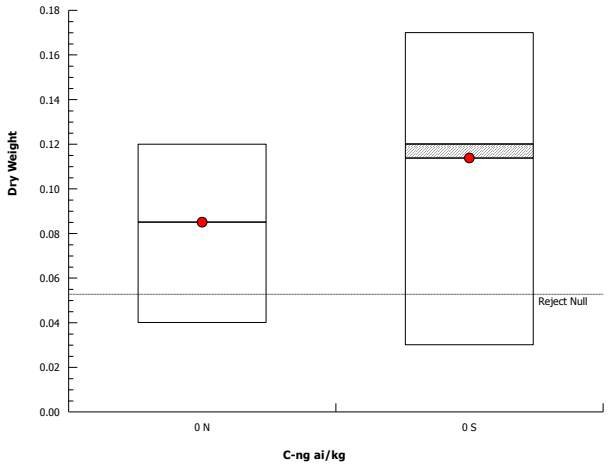
Distributional Tests

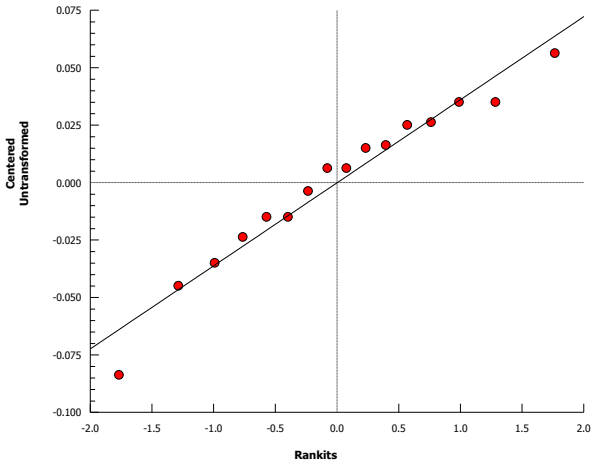
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.68	8.89	0.5082	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.963	0.841	0.7110	Normal Distribution

Dry Weight Summary

C-ng ai/kg	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	8	0.114	0.0794	0.148	0.12	0.03	0.17	0.0145	36.1%	0.0%
0	Negative Control	8	0.085	0.0586	0.111	0.085	0.04	0.12	0.0112	37.2%	25.3%

Graphics





OPPTS 850.1735 Sub-Chronic Sediment (10-d FW) **Wildlife International**

Analysis ID:	12-6953-3135	**Endpoint:**	Dry Weight	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Jan-15 15:49	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes
Batch ID:	08-8335-4172	**Test Type:**	Sediment Toxicity 10-d	**Analyst:**	
Start Date:	16 Jun-14	**Protocol:**	OPPTS 850.1735 Sub-chronic Sediment (1	**Diluent:**	Well Water
Ending Date:		**Species:**	Hyalella azteca	**Brine:**	Not Applicable
Duration:	NA	**Source:**	Aquatic Biosystems, CO	**Age:**	9d

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	129.0%	126	227	169.1	

Mann-Whitney U Two-Sample Test

Control	vs	C-ng ai/kg	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		85	31.5	NA	2	14	0.5274	Exact	Non-Significant Effect
		126	18.5	NA	4	14	0.9273	Exact	Non-Significant Effect
		227*	49.5	NA	3	14	0.0333	Exact	Significant Effect
		505	16	NA	0	12	0.8548	Exact	Non-Significant Effect
		804	8	NA	0	7	0.1111	Exact	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.03631186	0.007262372	5	2.42	0.0563	Non-Significant Effect
Error	0.09899583	0.002999874	33			
Total	0.1353077		38			

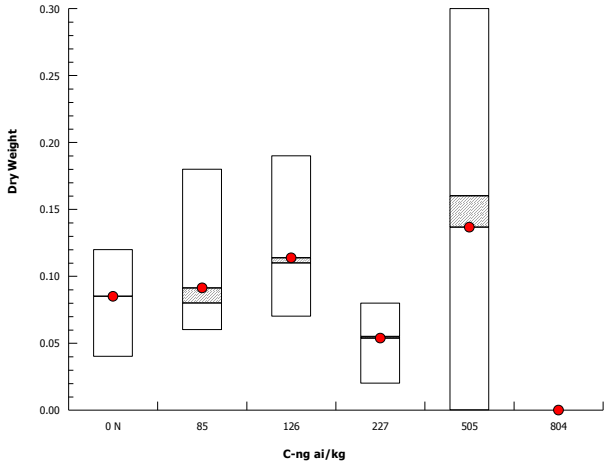
Distributional Tests

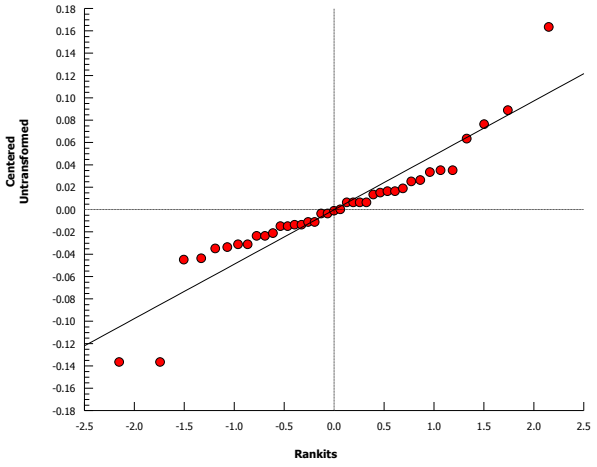
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Levene Equality of Variance	5.49	3.63	0.0009	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.894	0.922	0.0015	Non-normal Distribution

Dry Weight Summary

C-ng ai/kg	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	0.085	0.0586	0.111	0.085	0.04	0.12	0.0112	37.2%	0.0%
85		8	0.0913	0.0583	0.124	0.08	0.06	0.18	0.0139	43.2%	-7.35%
126		8	0.114	0.0831	0.144	0.11	0.07	0.19	0.0129	32.2%	-33.8%
227		8	0.0537	0.0383	0.0692	0.055	0.02	0.08	0.00653	34.4%	36.8%
505		6	0.137	0.0131	0.26	0.16	0	0.3	0.0481	86.2%	-60.8%
804		1	0			0	0	0	0		100.0%

Graphics





OPPTS 850.1735 Sub-Chronic Sediment (10-d FW) **Wildlife International**

Analysis ID:	14-9377-7985	**Endpoint:**	Survival	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Jan-15 15:33	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	08-8335-4172	**Test Type:**	Sediment Toxicity 10-d	**Analyst:**	
Start Date:	16 Jun-14	**Protocol:**	OPPTS 850.1735 Sub-chronic Sediment (1	**Diluent:**	Well Water
Ending Date:		**Species:**	Hyalella azteca	**Brine:**	Not Applicable
Duration:	NA	**Source:**	Aquatic Biosystems, CO	**Age:**	9d

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	8.95%	Passes survival

Equal Variance t Two-Sample Test

Control	vs	Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		Solvent Blank	0.266	1.76	0.083	14	0.3971	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.000625	0.000625	1	0.0707	0.7942	Non-Significant Effect
Error	0.12375	0.008839286	14			
Total	0.124375		15			

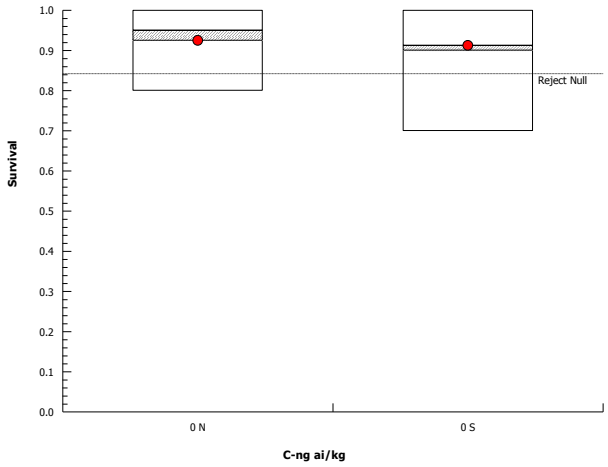
Distributional Tests

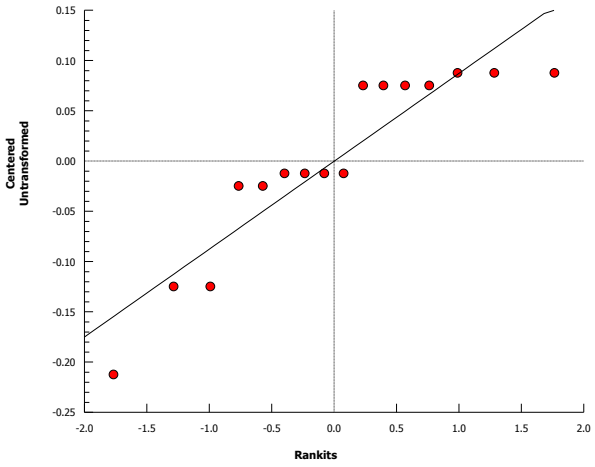
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.25	8.89	0.7760	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.843	0.841	0.0108	Normal Distribution

Survival Summary

C-ng ai/kg	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	8	0.912	0.83	0.995	0.9	0.7	1	0.035	10.9%	0.0%
0	Negative Control	8	0.925	0.851	0.999	0.95	0.8	1	0.0313	9.58%	-1.37%

Graphics





OPPTS 850.1735 Sub-Chronic Sediment (10-d FW) — **Wildlife International**

Analysis ID:	12-7546-8282	**Endpoint:**	Survival	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Jan-15 15:34	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes
Batch ID:	08-8335-4172	**Test Type:**	Sediment Toxicity 10-d	**Analyst:**	
Start Date:	16 Jun-14	**Protocol:**	OPPTS 850.1735 Sub-chronic Sediment (1	**Diluent:**	Well Water
Ending Date:		**Species:**	Hyalella azteca	**Brine:**	Not Applicable
Duration:	NA	**Source:**	Aquatic Biosystems, CO	**Age:**	9d

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	12.0%	126	227	169.1	

Mann-Whitney U Two-Sample Test

Control	vs	C-ng ai/kg	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		85	42	NA	3	14	0.1559	Exact	Non-Significant Effect
		126	31	NA	2	14	0.5490	Exact	Non-Significant Effect
		227*	63	NA	1	14	0.0002	Exact	Significant Effect
		505*	64	NA	0	14	<0.0001	Exact	Significant Effect
		804*	64	NA	0	14	<0.0001	Exact	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	6.7225	1.3445	5	84.3	<0.0001	Significant Effect
Error	0.67	0.01595238	42			
Total	7.3925		47			

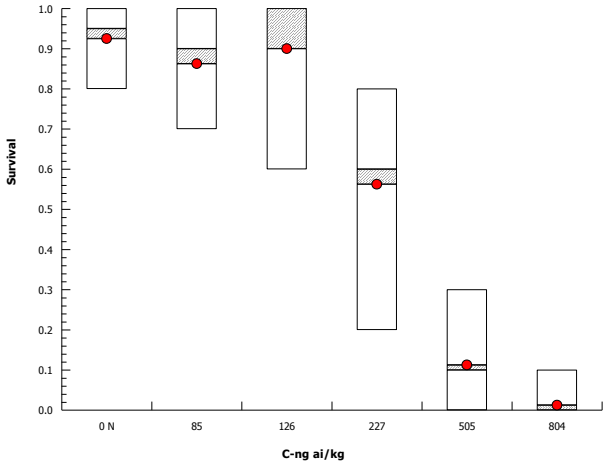
Distributional Tests

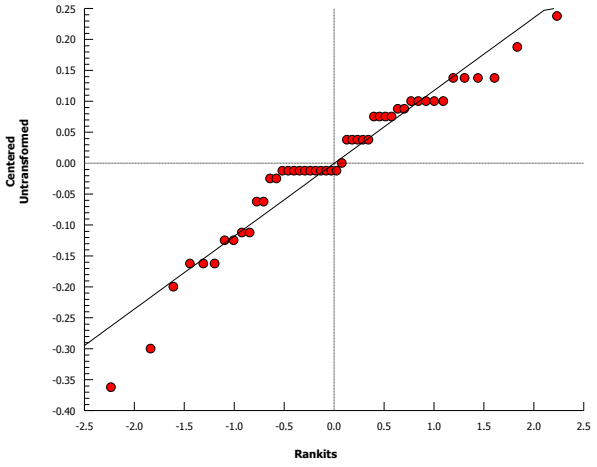
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	16.9	15.1	0.0047	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.945	0.934	0.0250	Normal Distribution

Survival Summary

C-ng ai/kg	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	0.925	0.851	0.999	0.95	0.8	1	0.0313	9.58%	0.0%
85		8	0.862	0.763	0.962	0.9	0.7	1	0.042	13.8%	6.76%
126		8	0.9	0.766	1	1	0.6	1	0.0567	17.8%	2.7%
227		8	0.563	0.402	0.723	0.6	0.2	0.8	0.068	34.2%	39.2%
505		8	0.113	0.0296	0.195	0.1	0	0.3	0.035	88.1%	87.8%
804		8	0.0125	0	0.0421	0	0	0.1	0.0125	283.0%	98.6%

Graphics





OPPTS 850.1735 Sub-Chronic Sediment (10-d FW) — **Wildlife International**

Analysis ID:	14-0975-2408	**Endpoint:**	Survival	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Jan-15 15:35	**Analysis:**	Nonparametric-Control vs Ord. Treatments	**Official Results:**	Yes
Batch ID:	08-8335-4172	**Test Type:**	Sediment Toxicity 10-d	**Analyst:**	
Start Date:	16 Jun-14	**Protocol:**	OPPTS 850.1735 Sub-chronic Sediment (1	**Diluent:**	Well Water
Ending Date:		**Species:**	Hyalella azteca	**Brine:**	Not Applicable
Duration:	NA	**Source:**	Aquatic Biosystems, CO	**Age:**	9d

Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	126	227	169.1	

Jonckheere-Terpstra Step-Down Test

Control	vs	C-ng ai/kg	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		85	1.13	1.64	4	-2	0.4886	Asymp	Non-Significant Effect
		126	0.0285	1.64	4	-2	0.4886	Asymp	Non-Significant Effect
		227*	2.95	1.64	5	-2	0.0016	Asymp	Significant Effect
		505*	5.13	1.64	8	-2	<0.0001	Asymp	Significant Effect
		804*	6.6	1.64	8	-2	<0.0001	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	6.7225	1.3445	5	84.3	<0.0001	Significant Effect
Error	0.67	0.01595238	42			
Total	7.3925		47			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	16.9	15.1	0.0047	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.945	0.934	0.0250	Normal Distribution

Survival Summary

C-ng ai/kg	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	0.925	0.851	0.999	0.95	0.8	1	0.0313	9.58%	0.0%
85		8	0.862	0.763	0.962	0.9	0.7	1	0.042	13.8%	6.76%
126		8	0.9	0.766	1	1	0.6	1	0.0567	17.8%	2.7%
227		8	0.563	0.402	0.723	0.6	0.2	0.8	0.068	34.2%	39.2%
505		8	0.113	0.0296	0.195	0.1	0	0.3	0.035	88.1%	87.8%
804		8	0.0125	0	0.0421	0	0	0.1	0.0125	283.0%	98.6%

Graphics